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SUBJECT:

CHARBON SYMPTOMATIQUE OF THE OX.

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PREFACE.

THE following article is a brief résumé of the facts connected with this disease. The following works have been consulted. Galtier's "Maladies contagieuses;" Arloing Cornevin and Thomas "Le charbon symptomatique du bœuf;" Williams "Veterinary medicine;" "Recueil de médicine vétérinaire;" "Journal de médicine vétérinaire and de Zootechnie;" Le repértoire de Police Sanitaire Vétérinaire and d'hygiene "Veterinarian," etc.

The reports which reach us from districts make no distinction between this disease and anthrax, and the consequence is extreme confusion. I am directed to ask that the two diseases be classified separately in future. In other countries more than half the charbonous diseases fall under the head of Charbon Symptomatique, and there is every reason to believe that this is the case in this country also. Information respecting the disease should be given as opportunities occur.

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MEERUT, 1894.

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CHARBON SYMPTOMATIQUE.

Nomenclature.—Charbon Symptomatique; Charbon bacterien; Emphysematous Charbon; Chabert's disease; Quarter ill; Black leg; Black quarter; Quarter evil; Strike; etc. etc.

Information is required under this head. The native names should be written in the character, and the English transliteration according to Hunterian system.

Definition and General Considerations.—Symptomatic or emphysematous charbon is a disease which has for many years been confounded with anthrax, and it is only a few years since it was shown to be an entirely separate disorder. It differs essentially from charbonous fever; and the difference of the nature of the two affections were foreseen and suspected by MM. Boulet-Josse, Vernant, Perroncito, Bollinger and Feser. The two latter scientists undertook a series of experiments in 1876-78 to establish the different nature of the two diseases, but it was reserved to MM. Arloing, Cornevin and Thomas to complete the study of the disease in a series of experiments carried out during the years 1879—1884. These observers have not only definitely distinguished the disease, but have also discovered its intimate cause, its pathogeny, etc.; they have observed that it does not recur; and have established methods of conferring immunity from it.

Charbon Symptomatique is an infectious, microbian disease, determined by a special anærobic, micro organism, and chiefly characterized by a hot, painful, then emphysematous and crepitant tumor which occurs in the muscular system.

Geographical distribution.—*Information under this head as regards India and Burma is urgently required.* Almost all the records to which we have had access contain statistics showing great numbers of deaths from Anthrax, but deaths from Charbon Symptomatique do not appear in them. We have reason to believe that very great annual loss of cattle from the malady occurs, and that probably a large percent-

age of the deaths attributed to Anthrax should be credited to this disorder.

The disease is common in Europe, Africa, America and Asia, in both hot and cold countries. It has been observed in North America United States, Central America, South America, Chili, India, South Africa, Algeria. In Europe it is more particularly prevalent in Switzerland, Germany, Austria, Italy, Spain, Belgium, Holland, and England. In France it is more or less general. It is not so common in Scotland as in England (Williams). It is prevalent at all seasons, but more particularly at the end of spring, in the summer, and at the beginning of Autumn; June, July, August and September are the worst months in France, Italy and Switzerland.

Information as to the season at which it is most prevalent in the various parts of India is required. It is more frequently seen in bovines than is anthrax.

It chiefly attacks young cattle, between the ages of six months and four or five years, and is most fatal in those which have rapidly come into condition. The greatest mortality occurs in animals aged between one and three years. Although sheep are not refractory, cases of the spontaneous disease are seldom seen amongst them.

Relative frequence of Charbon Symptomatique and Anthrax.—Hess has classified reports showing the following:—

First July to 31st December 1882.—Out of 286 cases of death from charbonous disease, 274 cases were due to Charbon Symptomatique and 15 to Anthrax.

From the 1st January to 31st December 1883.—Out of 721 deaths 642 were due to Charbon Symptomatique and 79 to Anthrax. Arloing, Cornevin and Thomas state that there can be no doubt that Charbon Symptomatique is by far more common amongst bovines than is Anthrax.

Symptoms of Charbon Symptomatique.—The disease attacks more especially animals between the ages of six months and four years, but not exclusively these. M. Hess gives the following analysis of 989 cases observed by him:—

374	cases in	animals	aged	between	6 and 12 months.
439	„	„	„	„	1 and 2 years.
83	„	„	„	„	2 and 3 „
65	„	„	„	„	3 and 4 „
10	„	„	„	„	4 and 5 „
18	„	„	„	„	5 and 6 „

With regard to the invasion of young animals by Charbon Symptomatique, M. Detroye quoted by Trasbot says: "The following observa-

tions have been made at the abbatoir at Limoges on this subject, and go to show that the disease is by no means so rare as is generally supposed :—

Date.	Seat of tumor.	Age of subject.
12th January 1890 . .	Hind limb . . .	2 months.
16th January 1890 . .	Fore leg and dewlap . .	2½ "
18th January 1890 . .	Right hind leg . .	2½ "
20th January 1890 . .	Left hind leg . . .	2 "
3rd May 1890 . . .	Right fore leg . . .	3 "
16th December 1891 . .	Both thighs . . .	4½ "
4th January 1892 . .	Shoulder	2½ "
16th February 1892 . .	Left thigh . . .	2 "
8th March 1892 . .	Shoulder	4 "

The existence of the disease in these animals was confirmed by experiment. M. Moulé states that out of 36 cases observed by him from 1886 to 1892 inclusive, 14 were in calves of about two months of age (Rec. de Med., Oct. 1892).

Animals which have rapidly come into good condition are more liable to it than are others. The attack is sudden, and occurs in two different ways; sometimes its existence suddenly reveals itself by the appearance of a tumor (charbon essential of Chabert), at other times more or less grave general symptoms precede the tumor (charbon symptomatique).

These symptoms consist of fever, derangement of digestion and arrest of rumination, partial tremblings of the quarters and shoulders, shivering fits, dryness of the muffle, dulness, inappetance, coldness of the extremities accompanied by lameness, the cause of which is at first hidden, but which we are able later on to attribute to the development of a tumour on one or other of the limbs. There is often apparently a remission in the symptoms after the appearance of the tumor, the animals feed and even ruminate a little, but the apparent amendment is not real, although it may lead the inexperienced practitioner and the owner to hope for a recovery, which will not take place.

The tumor may appear on any part of the body, be very marked, or hidden and impossible to demonstrate during the life of the animal. Generally it has its seat in those parts which are richest in masses of muscle.

Most frequently it appears at the superior parts of the limbs and in the neighbouring muscles, around the shoulder or the arm (*mal d'epaule*,

avant cœur, anticœur), of the croup and thigh (*mal de cuisse*), of the thigh (*mal de jambe*), of the genital organs (*trousse-galant*). In other cases it appears on the head, neck and trunk, on the masseters, in the submaxillary region (*estranguillon*), in the throat (*esquinancie gangreneuse*), along the jugular channel in the pectoral region on the sides of the chest, on the lumbar region or on the mammary gland. Hess has compiled a very valuable table showing in what parts the tumor most commonly appears, and from this we learn that the most frequent seats are:—right hind limb 13·51°/₀, right fore limb 10·86°/₀, left hind limb 9·24°/₀, right shoulder 7·18°/₀, left fore limb 6·33°/₀, left shoulder 5·94°/₀, chest 6·14°/₀, head and neck 5·04°/₀. Whatever its seat, this tumor is badly circumscribed and progresses in all cases with astonishing rapidity; in eight or ten hours it has acquired a great size. At first homogeneous and extremely painful at all points, it becomes by degrees insensible at its centre, crepitant and sonorous as a bladder filled with air. All the tissues which form it are black, friable and easily torn. On incision at the beginning of the disease it gives exit to thin blood, and at a later period to a liquid resembling venous blood, and at the end of the disease to a spumous serosity. When the tumor has its seat in a region very rich in connective tissue, however, it shows itself by a voluminous œdema, the fluid of which is citron in colour or slightly red.

If in the great majority of cases the symptoms unfold themselves as we have mentioned, such is by no means always the case. There are cases where dullness, dryness of the muffle, loss of appetite, some shivering, slight colic and tympany are the only objective signs; the diagnosis is very difficult in these cases, and we are likely to mistake it for some digestive derangement. The tumor, but slightly developed, may easily escape notice, even at the autopsy, runs its course in the depth of the muscular masses without attaining the superficial layers. We have occasionally found it in positions where we are not led to look for its appearance, such as the diaphragm. In cases where there is no sign of a tumor at the exterior of he body, we should search carefully at the autopsy in the thickness of the thoracic and pelvic muscles, remove the shoulder and examine the muscles which it hides. We are nearly certain to find some black or claret colored infarcts; whilst the tumor is being evolved, the general symptoms are exacerbated, the fever increases, the pulse is hard, beating 90—100—110 times a minute; the respiration is plaintive and accelerated; the temperature of the skin is much elevated, the animal becomes feeble and indifferent to its surroundings; the gait is painful and uncertain; the adynamic condition becomes more and more pronounced, the animal lies down and remains extended on the ground: the skin becomes cold, and death generally occurs in from 36 to 55 hours after the appearance of the

first symptoms. The oscillations of the temperature under the influence of this disease are very remarkable. The following gives a good idea of what occurs in the sheep :—

Wednesday evening	40·7°C. (3 hours after inoculation).
Thursday morning	40·4°C.
Friday morning	41·9°C.
Saturday morning	41·3°C.
„ at 2 p. m.	40·6°C.
„ „ 6 „	38·6°C. (an hour before death).

We have observed a maximum temperature of 42′ 8° which has been maintained for twenty-hours, after which it rapidly fell to 37° at death.

It has been said that a remission occasionally occurs after the appearance of the tumour, a respite during which the animal sought to feed and ruminate. The subjoined tracing, taken from M. Gotti, shows this very clearly. The temperature of the bullock, which furnished it, was 38·2°C at the time of inoculation, the next day it rose to 39 7°C and fell to 39·4°C, where it remained until the morning of the following day, when it rose to 40·5° and then fell to 38·8° just before death.

If we open the jugular during the course of the disease, we observe that the bleeding is not dribbelling; the blood which flows freely, rapidly coagulates and separates its serum more quickly than blood from a healthy animal.

Terminations.—The most common is death, many veterinarians never having observed a cure. Sometimes recovery does occur, and in these cases it is always preceded by considerable œdema about the seat of the tumor. At times death may result after a month or two has elapsed, from a metastatic pleurisy.

It appears that recoveries are not uncommon in Algeria, and probably they often take place in India.

Mortality.—The mortality caused by this disease is very great. Animals affected under two years of age almost invariably die, but older ones frequently recover; so much so is this the case that many affirm that all animals of over three years of age are free from danger of contracting the disease.

Post mortem Appearances.—The cadaver becomes rapidly distended with gas, and spumous blood flows from the mouth, nose and anus, due to the tympanitis.

Besides the gas which develops in the abdominal viscera, some is set free in the subcutaneous and intramuscular tissues of the part in-

vaded by the tumor. Thus, on raising the skin, we observe that the subcutaneous connective tissue is the seat of emphysema, more particularly about the shoulder and back, but also at times extending to the croup, quarters and thighs. We occasionally meet with a serogelatinous infiltration into the cellular tissue of various parts of the trunk. The muscles may be cribbled with hæmorrhagic effusion, and easy to tear; but what is most striking is the presence of one or several characteristic tumors on them.

These tumors vary as to their seat, as has been already stated; they may be confined to the deeper parts of large muscular masses, on the diaphragm, in the depth of the pelvic muscles or those of the thorax, under the scapula, etc., but more frequently they attack the superficial layers of the muscles of the regions where they exist. Besides these, we may meet with infarcts of black or claret color, of more or less considerable extent in certain of the muscles. We may observe one or several sanguinolent tumors more or less voluminous. The muscles invaded by the tumor are very dark colored, especially at their centres; but this coloration becomes modified as we leave the centre and approach the periphery passing successively from a very dark red to rose and yellow with black streaks. The dark color of the central portions becomes modified by exposure to the air, turns brighter and brighter in color after a few moments exposure. When the tumor exists in a part very rich in loose connective tissue, it is surrounded by a more or less voluminous œdema, of red color and strewn over with grains or yellow fibrinous filaments in the neighbourhood of the diseased muscles. In addition to the œdema, we observe a more or less marked gaseous infiltration more especially abundant when the œdema is small in quantity. The gas may be present in only small quantity in cases where death has been very rapid, as has been observed in guinea pigs dead from the inoculated disease; but it is generally present in tumors of the ox tribe, and is extensive in the inter and intra muscular tissue around the vessels and nerves. The parts infiltrated with gas are crepitant, elastic, less dense, and the muscular fibres are dissociated and friable. The gas gathered a short time after death is odourless, and is composed almost exclusively of carbonic acid with a trace of marsh gas.

Microscopical examination of a fragment of the œdematous tissue, which surrounds the tumor, reveals a fibrinous reticulum between the fascia of connective tissue, containing cellular elements and special micro-organisms. The muscular fibres easily dissociated are surrounded by masses of blood globules and leucocytes: certain of them, compressed by the escaped blood, by lymphatic cells, and fibrin, deprived of oxygen, have undergone fatty or colloid degeneration; some have here and there lost their structure, and present granulations which are stained black

with osmic acid ; others present fractures and appear as hyaline, vitreous and refringent blocks.

The characteristic pathogenic micro-organisms are abundant in the tumor; we find them around the fascia, in the lymph spaces of the intra muscular connective tissue. We find them just inside the sarcolemna of the muscular fibres, and more particularly at those spots where fracture of the fibres has occurred transversely. They are accompanied by migrated cellules and a certain quantity of fibrillated fibrin. The morbific agent of charbon symptomatique tends to provoke mortification of the muscular tissues in which it determines the evolution of a tumor. We may, in fact, cause localized mortification of the connective and muscular tissue by inoculating the virus of the disease into subjects which have been protected by inoculation or those which are refractory to the disease, which resist and allow the organisms to perform their work. In this way, by inoculating the virus into the muscle of a guinea pig, an ox, or a sheep, previously protected by inoculation, or into a soliped animal which has natural immunity, we give rise to a limited engorgement, which persists for a certain time, and which is nothing more nor less than a sequestrum composed of brown or yellow mortified fibres. This sequestrum becomes surrounded by a connective tissue capsule, the cellules of which progressively absorb its *débris*.

When the tumor invades the vicinity of a bone, the periosteum becomes thickened, red, presents blood extravasations between its fibres ; the marrow is vascularized, becomes redder, and presents micro-organisms. In the abdominal cavity of animals which have presented symptoms of colic, we find a more or less abundant deep colored serosity containing few blood globules, and some round micro-organisms as well as batonnets. When a tumor has extended to the abdominal walls, when a portion of the intestine is inflamed, we find claret colored spots on the peritoneum on a level with the diseased points. In certain cases the peritoneum is marbled with sanguineous effusions. Sometimes the abdominal muscles are black and studded with extravasations or infarcts ; at others they are pale, degenerated and friable, resembling cooked flesh.

Sometimes the epiploon and the mesentery are studded with ecchymotic patches (Delamotte).

When glos anthrax is present in charbon symptomatique, that is to say when we have tumefaction of the throat and tongue in this disease, the walls of the pharynx and œsophagus are black and friable, and these lesions may extend to the muscles at the base of the tongue and the soft palate. The intestine is found to be red, congested and studded with blood spots, and it is frequently thickened at parts. The walls of the stomach rarely undergo any change. The liver and spleen appear normal, undergoing no changes as to volume or consistence, and thus

markedly differing from what they show in charbon bacteridien; this enables us to distinguish readily between these diseases as a rule, but there are cases when a microscopical examination of the spleen pulp is absolutely necessary to enable a certain diagnosis to be made. Both these organs contain a considerable number of the pathogenic micro-organisms, which are also present in the bile. My own opinion is that we should seldom neglect to make a microscopical examination when it is practicable, as by doing so we demonstrate the "bacterium" if the disease be charbon symptomatique, and the "bacillus anthracis" if we are dealing with charbonous fever.

The kidneys are often normal in appearance, sometimes infiltrated with blood, owing to the presence of a tumor in the neighbourhood; they may occasionally be found to be congested and surrounded by serosanguinolent infiltration, which may or may not be mixed with gas. The pathogenic microbes exist in the kidneys and urine.

The scrotum is distended, and the testicles wine colored when the tumor is situated on the posterior extremities. The placenta is tumefied and gorged with microbes in the pregnant female.

In the thorax we meet with changes similar to those seen in the abdominal cavity, red plaques and hæmorrhagic patches in the lungs, serosanguinolent effusion when the tumor has invaded the pectoral walls. The lung is often hyperæmic. The respiratory mucous membrane, principally that of the anterior passages, sometimes shows deep congestion, and may be even black. The pleura often contains a sanguinolent serosity; the thoracic muscles are dark when the tumor has made its appearance on the shoulder.

The pericardium contains serosity, "the myocardium presents here and there hæmorrhagic patches; the endocardium is generally stained in both cavities, more particularly towards the summits. The blood which fills the cardiac cavities and the large vessels coagulates as in the healthy animal" (Arloing, Cornevin and Thomas).

The blood appears to have undergone little if any change either in its physical or anatomical characters; it is coagulable, and its globules are not deformed. It sometimes contains gas and organisms immediately before death.

The majority of the lymphatic ganglia are altered, more especially those near the tumor, which are hyperæmic, red and infiltrated. They are generally rich in microbes.

Etiology.—Charbon symptomatique is determined by a special micro-organism, which may be isolated, cultivated, and which, inoculated in the form of a pure cultivation, reproduces the disease. The affection is transmissable by inoculation to those animals which are susceptible to it, provided that we inoculate the virus in sufficient quantity and accord-

ing to a certain method. In practice the disease is almost exclusively observed affecting bovines, which become infected by introducing into their system with the food, drink or inhaling with the dust the pathogenic agents, which they get from external media.

Seat of the virus.—The virus is most abundant in the tumors; it is also found in other diseased organs, in the ganglia, liver, spleen, kidneys, etc., and in certain products of secretion and excretion. The pulp obtained by trituration or scraping of the black portion of the tumors is very rich in microbes: by agitating it with sterilized water we obtain an emulsion which, after filtration through linen, may be employed for inoculation purposes. The serosity or the blood-stained material which escapes from the incised tumor may also serve for inoculation; as may also the serosity of the œdemata, that from the serous membranes, peritoneum and pleura, etc., but the virulent agents are incomparably less abundant in them than in the tumor and the serosity surrounding it. The pathogenic microbes are always more or less abundant in the diseased organs, in the thymus, ganglia, spleen, liver, kidneys, lungs, mucous membranes, etc. It may pass, when hæmorrhage occurs, into the secretions and excretions bile, milk (Galtier), amniotic fluid, aqueous humor, semen (Galtier), excrement, urine (Galtier). The blood does not contain many microbes excepting towards the end of the disease, and after death they multiply and become abundant.

Nature of the virus.—The microbe which we find in the tumor, diseased organs and tissues, and in the blood at death, is the agent of virulence; it causes the disease. The following proofs clearly establish the rôle which it plays.

The liquid obtained by filtration of an emulsion rich in microbes is deprived of all pathogenic properties, whilst the part remaining on the filter produces the disease. When we dilute a virulent emulsion and leave it at rest for two or three days the superior strata contain few or no microbes and are not virulent; whilst the inferior strata, which are very rich in microbes, are also very virulent. When we provoke a tumor by inoculating a large dose of the virus into the connective tissue, the microbes only appear in the blood a few hours before death, and it is only then that this fluid becomes virulent. In like manner the urine does not become virulent until it contains microbes. The micro-organisms which are found in the lesions of the disease assume two different forms, as we shall see later on; we observe the bâtonnet and the spore form. By cultivating under the microscope bâtonnets provided with one or two brilliant bodies, we see them undergo degeneration and set free their spores; and these spores reproduce the bacillary form and determine the disease. In this manner blood taken from an animal at a time when it contains spores gives the same results as that taken from the tumor,

which contains the microbe in its various forms, and more particularly as bâtonnets. Lastly, the microbe taken from a sick animal and sown in artificial culture media, purified, isolated by successive cultures, reproduces the disease on inoculation.

The virus presents itself in several forms. As a bacillus, bâtonnet straight and mobile, formed of one or two articulated segments varying in length between 5u to 10u; it multiplies by scissiparity or segmentation, and also forms spores in the sick animal. In preparations made with the pulp of the tumor, for instance, we see many bâtonnets which contain spores in their interior. Those about to produce spores are irregularly swollen, sometimes in the whole of their length, assuming somewhat of a spindle shape, but more generally the spore appears at one of their extremities, causing a swelling, and giving to the microbe the appearance of a pendulum. The thickness varies from 1·1 to 1·3u, when they are spore-bearing. The same bâtonnet may sometimes produce two spores, one at each extremity, but this is not a common occurrence.

We find very much longer bâtonnets in certain organs; it is not rare to meet with them in masses.

In the tissue of the muscular tumor, the micro-organism assumes its most characteristic forms. We find it under the form of straight and regular bâtonnets; in the form of clubs, pendulum-shaped, or racquet-shaped with one extremity bearing a spore and enlarged; spindle-shaped, swollen in their middle with or without apparent spore; lastly of a rounded or ovoid form. We find them as bâtonnets, pendulum-shaped, and more especially as entire regular rods more or less elongated, in the bile, spleen, liver, kidneys, urine, ganglia, lung, etc.

In the blood we find them either as entire straight rods or rounded masses. To recapitulate, then, we may meet with the pathogenic micro-organism of this disease in three principal forms, *viz.*, in the form of entire and homogenous rods; under the form of spore-bearing rods swollen in their middle or at one extremity; and as rounded or ovoid bodies. The virus may be cultivated in an artificial nutritive medium; it is anœrobic. MM. Arloing, Cornevin and Thomas remark: "This organism is not easily cultivated." They have, however, succeeded in making cultures in broth. The details of cultivation need not be entered into here; it will be sufficient to say that a culture in veal broth or serum, made in an atmosphere of carbonic acid, preserved its virulence for three generations, after which it became attenuated, and it remained capable of giving immunity during the fourth and fifth generations, after which its activity appeared to become extinct. It may be cultivated in chicken broth, to which has been added a small quantity of glycerine and sulphate of iron, and in this medium the best cultures are obtained in vacuo.

The cultivation of the microbe in vacuo has succeeded with others, in the presence of inert gases. Galtier has essayed cultivations and has drawn the following conclusions from his experiments :—

Cultures made with blood, juice from the tumor or with any other product succeed when made in beef broth, gelatine or jelly. They develop well at the ordinary temperature of the laboratory, that is, at from 15° to 25°. They develop more rapidly in a stove regulated to 30°-37°C. They develop in vacuo, also in the presence of air when the broth is in deep layers and the seed is inoculated by deep puncture of the gelatine. Culture in broth in vacuo at 37°C increase very rapidly, after 20 hours the broth has become slightly lactescent. The preparations contain innumerable motile, round or ovoid microbes and others in the form of bâtonnets of various lengths. Later on a white colored deposit forms at the bottom of the tubes, and the broth becomes very limpid; when the tube is agitated the deposit becomes suspended as a white cloud of fine dust. These cultures, even those which have not been made in vacuo or in the stove, cause the disease when inoculated into the guinea pig. Cultivated in gelatine and jelly, the microbe gives rise to the formation of gases; gelatine is liquified in three days, and in from 20 to 22 days all the medium is nearly completely liquid, the microbes forming white masses which fall to the bottom of the tube. The microbe of charbon symptomatique which MM. Arloing, Cornevin and Thomas have named "Bacterium Chauvœi" in recognition of the services which M. Chauveau so kindly rendered them during the period of their studies, and the classification of which is still not certainly determined, is a bacillus or a bacterium which stains with the dyes used in the preparation of various bacteria. It resists the action of acids and alkalies; acetic and more especially formic acid clears it. The pulp scraped from a section of the tissues of the tumor and mounted, shows numerous microbes easily seen without the aid of any stain. But preparations previously stained show the microbe best. The microbe may be stained with gentian violet fuchsine, methylene blue, etc.; it stains well with Löffler's blue (potass 8—10,000, alcoholic solution of methyl blue 1); with hydro-alcoholic solutions and especially with aniline solutions of gentian violet, fuchsine, etc.; it resists decolorization by Gram's solution; it is not decolorized, when heated in balsam, when it has been stained with aniline. We may obtain beautiful stained specimens, with the serosity or virulent liquids, or with scrapings or pulp from diseased organs, as well as from diseased tissues previously hardened in alcohol, by using the above mentioned stains.

Slips or cover glasses furnished with pulp culture, or sections are treated as for staining the bacteridium of Davaine "Bacillus anthracis;" leave them for ten to twenty minutes in the coloring material, then

wash, dehydrate, clear and mount in balsam. In such preparations we distinguish easily the peculiarities of form assumed by the microbe; those which are in the form of rods appear uniformly colored throughout their extent, etc.

Resistance of the microbe to physical and chemical agents.—The virus resists dessication; we may obtain a very virulent material by drying the pulp of a charbonous tumor at 35° C. This resistance may be attributed to the resistance of the spores, as in anthrax. The dried virus may preserve its properties for years, and is more resistant to physical or chemical agents than is the fresh virus.

Very intense cold has no apparent effect upon the virus, which may be exposed to a temperature of —130°C without undergoing either sterilization or even attenuation. Fresh virus sealed in a tube may be kept for thirty minutes in a stove at 65° C without losing its activity; at a temperature of 70° C it is sterilized in two hours and twenty minutes; in two hours at 80° and in twenty minutes at 100° C. The addition of boiling water in the proportion of three parts to one of the virus does not sterilize it, whilst fresh material enclosed in a tube and left in water at 100° is sterilized in twenty minutes. Virus dried at 35° C bears a more elevated temperature; previously moistened or mixed with water and placed in the stove it withstands a temperature of 85° for six hours without undergoing more than a slight attenuation; heated for the same time at 90,° 100° and 105° C it undergoes more marked attenuation without losing its virulence, which is only destroyed by six hours' exposure to 110° C. The same virus dried and placed in a tube and put into boiling water is sterilized only after two hours' exposure. In a medium saturated with watery vapour heating to 110° C for fifteen minutes has sterilized the fresh virus contained in tubes, fresh and even dried virus previously mixed with water and spread over fragments of tissue: the dried virus added to water and enclosed in tubes has required heating for thirty-five minutes. The fresh virus, falling into a large quantity of water, becomes attenuated by such dilution; when it is mixed with a small quantity of still water, the microbes fall to the bottom of the vessel, and the superior layers are innocuous.

Putrefaction does not destroy the microbes rapidly in buried carcasses. They have been found to be virulent six months after putrefaction has commenced, but innoculation has failed to produce the disease when carried out thirty months after putrefaction has started, but this does not prove that the microbes have been destroyed. The microbe may live in company with others; we have produced at the same time charbon symptomatique and septicæmia, charbon symptomatique and anthrax; the germ is not destroyed by admixture with the ferments of rennet nor that of ammoniacal fermentation.

All these facts lead us to the conclusion that it may be preserved in the soil, and direct experiments have established the fact. *Feser* has caused the disease by injecting the microbes found on the plants from marshy pastures of the Alps, where the disease is rife. *Gotti* caused the disease by injecting the water, in which had been washed earth from San Arcangelo, where the disease had been prevalent, under the skin of young oxen It is apparent, therefore, that the germs may be preserved in the soil or on its surface, and this explains how it is that animals which live in places where these germs exist, may ingest them.

The virus offers great resistance to those chemical agents which we designate bactericides; it is only sterilized, especially when dry, by the most energetic of these. Solutions of potash, lime, sulphate of iron, sodium chloride, arsenious acid, chloride of zinc, turpentine, sulphurous acid, etc., are useless for purposes of disinfection. The agents which may be recommended for this purpose are:— phenic acid in aqueous solution 2-4°/₀; salicylic acid 1-2°/₀; nitrate of silver 1-2°/₀; sulphate of copper 1-5; perchloride of mercury 1-2 p. 1,000, etc.

Animals susceptible to the disease.—Charbon symptomatique is nearly entirely confined in its attacks to the bovine species; it has only exceptionally been witnessed in young sheep and solipeds. It is transmissable by inoculation to oxen, sheep, goat, and guinea pig. The carnivora, pig, rabbit and bird are refractory. The disease does not appear to be transmitted to man.

The large and small ruminants are the most susceptible to the disease, although, as has been stated, oxen do notexhibit the same receptivity at all ages. The spontaneous malady is very rare in calves before the age of six months, and it is rather a difficult matter to induce it in suckling calves. Well marked and characteristic cases have, however, been noted by veterinarians in young calves, which had already been submitted to a vegetable diet. This *quasi*-immunity which young calves enjoy appears to be due partly to heredity transmitted by an already immune mother, and more particularly to the mode of alimentation special to the age, as well as to the condition of the tissues and humors during that age. Experimenting with animals of various breeds, we have been able to inject six drops of fresh virus of a very active character into the muscles of the young with impunity, whilst animals more advanced in age succumbed in the proportion of 90°/₀ under similar conditions. These inoculations, however, sometimes give rise to lameness and elevation of temperature in certain of the calves; and by employing larger doses we cause death. Six or eight drops may destroy calves of less than sixteen days of age; it requires from ten to twenty drops to kill calves aged from fifteen days to three months. The

feeble receptivity of the calf is very evident from its behaviour to the intravenous injection of strong virus and the subcutaneous injection of attenuated virus which give to the adult animal a mild attack and confer immunity, whilst the calf has no protection conferred upon it by the proceeding. The receptivity of young calves increases as they become more entirely herbivorous. The influence of diet is still further shown by what occurs in the young guinea pig, which is very susceptible to the disease, because it becomes herbivorous very early after birth.

Oxen from localities where charbon symptomatique is enzootic are not apt to contract the disease after they have passed the age of four years.—This has been demonstrated both by clinical observation and by experiment. But this privilege is not enjoyed by adult animals, which have been raised in regions where the disease is not endemic; these animals contract the disease at all ages when they are inoculated, or when they are introduced into localities where it is rife. Immunity is then only acquired by animals (adult) which have been reared in localities where the disease is endemic; and this immunity appears to result from successive inoculations, either with weak doses of the virus, or with germs which have become attenuated in external media which occur spontaneously during the youth of the animals. It may be likened to what occurs when we inoculate small doses of the virus or attenuated virus Young cattle living in an infected area may ingest or otherwise introduce into the system variable doses of germs more or less attenuated. Some in this way become inoculated with sufficiently large doses to provoke a fatal attack; whilst others, by receiving either attenuated virus or a small dose of the virulent, only contract a mild form of the disorder which may pass unperceived and confer on them relative immunity, which may be increased by further spontaneous inoculations, and become more or less absolute at the age of four or five years, or even before.

The receptivity of the sheep is very marked; and, although it seldom contracts the disease spontaneously, it may be easily infected by inoculation. As to the goat, it may also contract the disease after inoculation, but less surely than does the sheep. Lastly, the guinea pig may be employed as a soil for the culture of the virus. Its receptivity is far from equal to that of the ox and sheep; the virus tends to decrease in virulence when it is passed from pig to pig, and to such an extent does this weakening process occur that after a time we obtain such an attenuated form as to be capable of conferring immunity without danger.

The horse and ass, at adult age, are generally refractory, resisting inoculations and only presenting local lesions as the result of them. Clinical observation has shown or led us to suppose that foals are more susceptible and may even contract a grave form of the disease.

Amongst the refractory animals, one may be rendered susceptible to the disease, and this is the rabbit. When we associate the virus of charbon symptomatique with another microbe, especially the micrococcus prodigiosus (Roger), we may induce the disease in this animal by inoculating the mixture. The "prodigiosus" favours infection by the action of its soluble products, which modify the general state of the animal, when they pass into the circulation. This is proved by the fact that the receptivity is more especially increased when the prodigiosus is injected directly into the circulation. The receptivity created is evanescent, disappearing in twenty-four hours. This fact is interesting, as it shows us that a microbe, inoffensive in itself, may create a passing receptivity to a morbific one in subjects ordinarily refractory; and that it is not impossible that animals more or less refractory may in practice be rendered susceptible to a grave form of the disease.

In addition to all this the microbe of charbon symptomatique, which is a ferment liable to induce butyric fermentation in the majority of sugars and of several neutral hydrocarbons (Arloing) secretes soluble matters (Roger) which favour its development. Hence, when it is inoculated into the anterior chamber of the eye of a rabbit, it causes the disease and death, although this is not the case when it is inoculated into a muscle or under the skin. The soluble substances secreted in the eye modify the general health of the animal and create receptivity; the absence of phagocytes in the aqueous humour explains the development of the microbe in the eye. More than this, serosity obtained by filtration of virulent material injected in doses varying from 1 to 5 C. C. into the vein of a rabbit, temporarily abolish its natural immunity and render an inoculation into the muscles successful; the predisposition so created does not last more than twenty-four hours. M. Roux has demonstrated, as we shall see later on, that such serosity has vaccinal properties If the prodigiosus and the substances of *bacillus chauvœi* produce temporary predisposition in the rabbit, this arises from a momentary weakening due to the action of these substances, which render the animal more or less ill, and consequently less resistant for a time. This explanation of the phenomenon appears so probable that MM. Nocard and Roux, by enfeebling the vitality of a muscle with acetic acid, potash, alcohol, etc., or by producing a traumatism, or a contusion, have been able to render the rabbit susceptible to the disease, and the virus has become intensified and more active to the normal rabbit. Galtier himself has succeeded in inducing charbon symptomatique by inoculation in the rabbit which he had weakened by feeding it on watery food and by submitting it to the action of cold. These facts are interesting from a clinical point of view, as they show us clearly some of the predisposing causes of the disease.

Modes of Contagion.—Influence of the dose and the mode of penetration of the virus.—In a general way, the grave form of the disease is only produced by a more or less large dose of the virus, which varies according to the manner and the seat of its introduction, and of course the degree of its activity; punctures with a lancet are not usually successful in inducing the disease for the reason that they do not permit of the introduction of the virus in sufficient quantity nor to a sufficient depth; the inoculated virus must be placed in the subcutaneous tissue if we wish to be certain of its producing its effects, at the same time certain regions are not favourable for this method of inoculation, if we wish to induce a fatal form of the disease. Thus, the tumors do not develop below the knee and hock, nor in the tail: and the virus inoculated in doses of one to seven drops at the extremity of the tail determines neither local nor general disturbance. Injected in doses of ten drops it causes elevation of temperature; injected in very large doses (two grammes) it gives rise to a local engorgement, fever, loss of appetite, rise of temperature, and confers immunity. If injected at twenty centimetres below the root of the tail it is more dangerous, and may, exceptionally it is true, cause death in from three to four days, the virus taken up by the lymphatics or blood vessels is transported to other parts of the body and there produces at some part a fatal tumor. In proportion as we approach the base of the tail the chances of general infection and the formation of symptomatic tumors increase, but do not equal those following inoculation into the thigh, quarter, or neck. The tail then possesses a feeble receptivity, which increases as we approach the root; this is due to the low temperature and the density of this region.

The most favourable seats for inoculation are those rich in muscle, or in which connective tissue is abundant and lax, and the temperature fairly high. The virulent material for inoculation may be extracted from various sources: from all the lesions and products reputed active as well as from cultivated microbes. In order that we may be certain of the result, material from the ganglia, organs rich in microbes, and especially from the tumor, should be employed.

Virus taken from the tumor may be used either in the fresh or dried state (dessicated at 35°C.) The material to be employed is obtained by triturating fragments of tissue taken from the blackest portion of the tumor, in a glass or mortar; to this is added sterilized water, and the whole is shaken in a flask and filtered through linen; the filtered material serves very well for inoculation. The dried material is obtained by submitting the pulp of the muscle invaded by the tumor to rapid dessication, selecting those portions of it which have undergone most morbid change. The method adopted is first to mince the material; add

sterilized water; bruise in a mortar or shake well in a flask; filter and spread the material on plates and place in a stove regulated to 35°C. After complete evaporation and dessication, a bright reddish layer of material remains, which is to be reduced to powder and may be kept in a flask dry and in a dark place. Virus so prepared may be kept for more than five years. When we desire to use this virus for inoculation, it is necessary to add a little sterilized water to it and triturate in a mortar and filter through calico; the virulent liquid thus obtained may be increased in virulence by adding a drop of lactic acid.

Inoculation with a lancet, as has been already said, is not a satisfactory method with this virus; it does not produce any local lesion, or tumor when made in the ear of a guinea pig; although it may induce a fatal attack of the disease. When this occurs the animal dies in about three days; at the autopsy we find infarcts in the muscles of various regions, and congestion with tumefaction of the lymphatic ganglia. The most certain method of inoculation is the subcutaneous, the injection into the subcutaneous connective or the intra muscular tissue. The effects of the inoculation vary according to the dose employed and the region selected. The dose of the virus injected should generally be large; two or three drops injected under the skin of the quarter, shoulder, etc., suffice to induce fatal charbon in the sheep and oxen, whilst a similar quantity injected into a region rich in cellular tissue, such as the ear, extremity of the tail or limbs, is insufficient to do so. When the injection of virus has been made into the thigh of a guinea pig, the part becomes swollen and painful in a few hours; movement is impossible, and death ensues in from twenty-four to forty-eight hours. At the autopsy we find very extensive red coloured œdema of the connective tissue of the abdominal wall; a charbonous tumor at the seat of inoculation, with swelling, dark or black colour of the muscles, and a very marked smell of lactic acid pervading the parts. When a large dose of the virus is injected into the subcutaneous connective tissue or the muscles of an animal endowed with great receptivity, such as the sheep and ox, grave pathological changes occur and death results. If the injection has been made in the subcutaneous connective tissue, the lesions appear in the connective tissue and ganglia, encroaching little on the muscles; œdema, which is hot, firm increases rapidly and becomes crepitant in certain parts. When the injection has been made into the muscle, a true crepitant tumor is rapidly evolved analogous to that of spontaneous charbon, and accompanied at times by tumors in other regions.

The subcutaneous injection of medium doses ($\frac{1}{10}$ of a drop of pulp) produces effects similar to those following inoculation with the lancet; when successful the local lesions are *nil* or little marked, but muscular

lesions, a tumor more or less distant from the seat of inoculation, following the same course as those evolved during the course of the spontaneous disease, makes its appearance and is followed by death.

The injection of less active virus, or of a very small dose of active virus is followed either by no result, or by the appearance of slight symptoms, such as fever, loss of appetite, and dulness, without any local lesion; this slight derangement, however, gives immunity to the inoculated animal. In certain cases, however, where the animal inoculated is more than ordinarily susceptible, which we are unable to foresee, a virulent and mortal attack of the disease may occur from a very minute dose of the virus.

The introduction of relatively large doses of virus directly into the veins is usually well borne. The intravenous injection of doses varying from $\frac{8}{10}$ to 5 drops up to 6 C. C. of filtered muscular juice induces in oxen, sheep and goats a mild form of the disease (unaccompanied by tumors) with dulness, elevation of temperature, etc., lasting for two or three days and followed by immunity. Whilst practising intravenous injection it is of the greatest importance to avoid inoculating the perivenous tissue, as, should this accident occur, it will in all probability be followed by a virulent form of the disease. Sometimes intravenous injection with large doses, or with active virus, or when the animal is a very susceptible one, may be followed by a virulent form of the disease with all its symptoms.

Moreover, when very large doses are injected, the animal may succumb before the appearance of the tumor and without muscular infarcts; in these cases the ganglia are virulent. How are we to explain the reason why intravenous injection does not produce the disease with its characteristic tumor? Beyond the fact that a certain dose of the virus may be destroyed by the blood, we have reason to believe that if the microbes multiply in that fluid, and we know they do since it becomes virulent, they are retained by the vascular endothelium, which prevents their extending into the connective tissue, and hence the tumors are not formed. It is certain that if we permit them to emigrate from the vessels, they give rise to a tumor and cause death. Thus it happens, if during the slight malady which follows intravenous injection, we provoke a small hæmorrhage in any region, a tumor develops and the animal perishes. It appears, then, that in order to cause a tumor and induce a grave form of the disease, the microbe must gain exit from the vessels and undergo certain changes in the connective tissues. It appears also that there are two phases in the evolution of the disease—one the multiplication of the microbe in the blood, and the other intoxication when the microbe passes into the connective tissue; the virus remains in part and

is evolved in the tissue, and is in part taken into the general circulation by the capillaries and lymphatics; if only a small quantity of it remains in the connective tissue the local accidents are insignificant; if much passes into the blood, a tumor here and there may result; if much virus remains in the subcutaneous tissue, a tumor is produced; when death does not occur too rapidly symptomatic tumors appear, due to extravasations.

Intertracheal injection may give a mild form of the disease and immunity. The microbes injected into the respiratory passages pass from the infundibula into the vessels, traversing the pulmonary epithelium and the vascular endothelium. Attempts made to cause the disease by gastric ingestion are generally unsuccessful. Occasionally the injection of virus, intensified by methods which will be noted later on, has caused virulent charbon with tumors in the ox. It is certain, then, that animals may infect themselves by eating and drinking materials soiled with the virus.

Charbon symptomatique may be transmitted by intra uterine contagion when it attacks the mother during gestation; the mother, which has gained immunity, appears capable of transmitting it to her offspring. Animals which contract the spontaneous disease may infect themselves in various ways. The transmission occurs more particularly by germs derived from the sick or their cadavers, which have been distributed in external media and preserved intact. It may occur through the intermediary of soiled instruments, etc., which may be brought into contact with a wound on the skin or mucous membrane. We have seen bulls thus inoculated during castration. It may occur by the inhalation of dust mixed with germs. It may occur also by the ingestion of food and drink soiled with the virus.

When the microbes penetrate in sufficient quantity by a puncture or wound of the skin or mucous membrane, the disease begins with local lesions; a tumor forms. When the virus is introduced in small quantity into the connective tissue; when they gain the blood after inhalation or ingestion, the disease commences with general symptoms, and symptomatic tumors appear at a later date. In certain countries where charbon symptomatique is enzootic, it has appeared as though inundations favored infection. The air may be contaminated by dust bearing the virus. It is very fortunate, however, that animals which ingest, inhale or become inoculated with the virus of this disease only fall dangerously ill, in small percentage. This is because the microbes may be too weak, more or less attenuated, so that animals infected with them are only attacked by a mild form, and acquire immunity more or less complete.

Immunity.—Attenuation, recuperation, and exaltation of virulence—Vaccination.—A non-fatal attack of the disease confers more or less complete and durable immunity, and this is, if anything, more permanent as the affection has been grave. Such a mild attack may be induced by the inoculation of minimum doses of strong virus, by inoculating in certain regions (ear or extremity of the tail), by injection into the vein or the trachea, by the use of attenuated virus or soluble substances obtained from the microbe. Immunity may be heiditary, transmitted from parent to offspring, and, lastly, animals may acquire it by spontaneous infection with small quantities of, or attenuated, virus. The refractory state of conferred or acquired immunity implies that the animals which possess it are endowed with a bactericidal quality of their humors and a peculiar condition of phagocytosis of their elements powerful enough to destroy the microbes. All the elements of animals which are immune appear to be endowed with bactericidal powers. Roger has observed that the thigh of a vaccinated guinea pig does not form a suitable medium for the development of the virus when inoculated and placed in an incubator, whilst that of a non-vaccinated one, under similar conditions, soon develops a tumor.

Attenuation of virulence.—Exposed to the influence of physical or chemical agents, the microbes of charbon symptomatique may be progressively deprived of their activity before being completely destroyed. It is certain that the microbes in the soil undergo more or less rapid and important modifications according to its depth, composition, and physical condition. It is said that virus in the superior layers of the soil, where it does not undergo dessication, becomes rapidly attenuated under the influence of light and air. On the contrary, virus deposited at the depth of 1 metre or lower is preserved for a longer time. *Gotti* has been able to communicate the disease with the washings of earth from a depth of 0·40m. of a place where charbon symptomatique had not shown itself for several years. Germs so preserved may be brought to the surface by the same agents as have been mentioned under charbon.

When the microbes are cultivated in artificial media they may lose their virulence more or less rapidly, passing through various phases of attenuation, during which we may collect certain cultivations which may be used as vaccines. However the experiments which have been published are too few on this point, and we are hence inclined to be reserved. We cannot so far attach much weight to attenuation by means of antiseptic substances.

Attenuation by heat merits some consideration. We have already seen that it influences to a certain degree both the fresh and the dried virus. By heating the fresh virus from 40 to 70 minutes at 65°C., we

cause a certain amount of attenuation, which is evidenced by its action on guinea pigs being lessened. By exposing it in a sealed tube to a temperature of 70°C., for an hour and forty-five minutes, we may transform it into a vaccine. *Nuroletti*, by heating the virus in hermetically sealed flasks at 35 to 38°C., for several months, has produced attenuation, and rendered it vaccinal. *Kitasato* has conferred immunity on the guinea pig by injecting a culture of fifteen days, and by the injection of the virus which had been heated to 80°C., for thirty minutes. It is by the action of heat upon the dessicated virus, however, that we obtain the most uniform results. The temperature must be taken above 60°C. to obtain any certain attenuation, and experience has demonstrated that we should take it above 80°C. We obtain a very attenuated virus for premier vaccine by heating to 100 to 104°, and a deuxieme vaccine by heating to 90° to 94°; leaving the virulent matter in the incubator for seven hours, placing the material so attenuated in blotting paper and in a dry dark case in the presence of some deliquescent material, under which circumstances it is preserved for a year. Each vaccine is pulverized in a special mortar, sifted and preserved as a powder. The virus attenuated by heat, as has been indicated, does not transmit it to that derived from it in the sick animal; it regains noxious properties.

Kitt demonstrates that it is possible to confer immunity by one inoculation of dessicated virus which has been submitted to the action of steam at 100°C.

Recuperation and augmentation of the virulence.—The virulence of an attenuated virus may return to its original intensity, or even become augmented. We may revive its pristine activity either by cultivating it in animals very susceptible naturally or rendered so by artificial means, by traumatism or by the action of some substance on it. Thus, virus attenuated by seven hours heating at 100°C., which does not destroy the adult guinea pig, but is still virulent for the young newly-born pig, may be regenerated by inoculating it into this animal. Thus, also, by inoculating large doses into adult animals we may destroy them, and in this case we shall find that the microbes have regained their virulence.

MM. Arloing and Cornevin have given back to an attenuated virus its original virulence by leaving it for some hours in contact with one-fifth solution of lactic acid: "It suffices to add lactic acid to the water in which we dilute the virus and leave the water so acidulated in contact for five or six hours. After this time, inoculation with the virus previously attenuated communicates virulent charbon with certainty." Moreover, lactic acid intensifies the virulence of strong virus when it is employed alone or in the following manner: "If we mix lactic acid with

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a small quantity of water to which has been added some very fermentescible sugar and pour this into the virulent liquid thirty hours before inoculation, we obtain a virus of maximum intensity."

We have, therefore, in lactic acid an agent which intensifies the virulence of the microbe, and which is useful in demonstrating the presence of the microbes in the soil.

MM. Nocard and Roux have verified the above conclusions, and offer the following explanation of them: the virus attenuated by heat is not properly speaking a truly attenuated virus, for it cannot reproduce attenuated virus in culture; dessication and heat in the preparation of attenuated powder only leave the spores alive, render their power of germination and their action on the cells of the organism less; but anything which diminishes the vitality of the tissues renders the development of the virus easier, and seems to increase or restore its virulence. Lactic acid does not in reality exalt the virus; but, being injected along with it, modifies the cells of the organism and so increases the receptivity by diminishing the resistance. Experiment has shown that "a prolonged contact of the acid with the virus is not necessary to regenerate its virulence; the virus inoculated soon after its admixture with lactic acid shows itself to be as active as the other." Moreover, we may inject the virus and the lactic acid solution separately, and the effect is the same.

Protective inoculation.—We can confer on animals immunity from this disease, by inoculating the strong virus, the attenuated virus or the soluble products secreted by the microbes.

As we have already seen, we may cause a mild form of the disease, which is followed by immunity, by injecting a weak dose of the virus into the connective tissue of certain regions, into a vein or the trachea, or by injecting a very small dose into the connective tissue of any part. None of these methods, however, are used in practice. With regard to subcutaneous inoculation, there is too much danger. Subcutaneous inoculation at the extremity of the tail may be carried out with ease and may succeed in conferring immunity without danger, but inoculation with attenuated virus is preferable. As to intravenous and intertracheal injection, there are so many difficulties attendant on their practice, owing to the danger which must always exist of soiling the surrounding connective tissue, and thus, as we have already seen, inducing virulent disease, that we are fain to discard this method (see Robertson and Pemberthy Veterinary Journal, 1888). Robertson did not succeed in obviating the attendant dangers in his experiments. In case, however, that it may be desired to practice it, we give the mode of procedure here. Either fresh or dessicated virus may be employed, and it suffices to inoculate small quantities $\frac{3}{10}$ths of a drop of fresh virus for the

sheep three to ten drops for the ox. The dessicated virus is prepared as for vaccines, which will be mentioned later on. We obtain the fresh virus by killing a guinea pig with the disease, take a certain quantity of the tumor, triturate in sterilized water, filter through calico, shake the emulsion so obtained each time that we use it. If it be suspected that the virus is mixed with septic microbes, we may treat it with sulphuric acid, which rapidly destroys the latter, but has no action on the virus. With regard to the method of inoculation, it is the same as that adopted for pleuro. We have mentioned above the method of attenuation by heat, which gives us two vaccines of unequal intensity, and which may be used successively with a few days interval, commencing of course with the weaker.

The virus, attenuated by heat, gives a mild form of the disease on inoculation with a more or less considerable dose. To confer immunity on animals without exposing them to grave attacks, we make two successive inoculations—the first with virus attenuated by heating for seven hours at 100°—104°C., and the second with virus heated for seven hours at 90°—94°C. These vaccines may be used for guinea pigs or for large or small ruminants. The injections in oxen should be made into the subcutaneous tissue at the end of the tail, towards the centre of the tuft, or in the external surface of the ear as far as possible from the base.

The vaccines being in a state of powder must be subjected to certain manipulations before injection. A quantity sufficient for the inoculation of a certain number of animals, ten for example, is triturated in a mortar with 5 C.C. of sterilized water gradually added; then filtered through linen, which has been sterilized and moistened with sterilized water; we thus obtain 5C.C. of virulent emulsion, sufficient to inoculate ten animals. The syringe (pravaz) is filled ½ cubic centimetre (or even six to eight drops to animals under a year) is injected, shaking the material between each injection to distribute the microbes uniformly throughout the fluid.

Subcutaneous injection at the tip of the tail or in the ear is a most simple operation.

The seat of inoculation is cleansed, the hair removed, and the external face of the tail selected. The organ is seized in the left hand and with the right the canula introduced piercing the skin obliquely and the injection made; protective inoculations should be made preferably in the end of winter or the beginning of spring, eight or ten days should elapse between the inoculations. We may inoculate young oxen a few months after weaning—one month according to Delamotte. We may dispense with the inoculations when animals have been born or reared in a country where the disease is enzootic. We vaccinate more especial-

ly animals aged from six months to three years. There seems to be no danger in inoculating pregnant females, but it is prudent to avoid it if possible. Cows vaccinated whilst in calf may transmit immunity to their calves; and the calves from mothers vaccinated sometime after conception are more resistant than others.

The immunity resulting from inoculation appears to last about eighteen months, and it is advisable to re-inoculate young animals after a year's interval, whilst this is not necessary when the animals are vaccinated when aged about two years.

In order to prove the efficacy of protective inoculations, we may inoculate vaccinated and control animals with virulent virus, and compare the results. It will then be found that the vaccinated resist, whilst the non-vaccinated do not. It may occasionally happen that a tumor devolops in vaccinated animals, but it is seldom followed by death.

The effects of the preventive inoculations are mild. There is sometimes slight fever; the local lesions consist of a hot, painful swelling, which soon disappears. Sometimes an abscess forms, which may either terminate simply or may lead to necrosis of the tip of the tail.

Occasionally, though rarely, attenuated virus may lead to virulent charbon, but this fatal result does not occur more than once or twice in a thousand animals, however it shows us how necessary it is for the veterinarian to omit no precaution.

Since the year 1881 numerous proofs have been accumulated which establish the economic value of preventive inoculation. The inoculations with virus attenuated by heat have been adopted in a number of countries. They have given the most satisfactory results and have diminished the mortality in the various departments of France, in Switzerland, the Tyrol, Germany, Belgium, Italy, Austria and America; everywhere, thanks to protective inoculation, we have seen the mortality almost completely cease its ravages amongst the inoculated animals, whilst it has continued in the non-inoculated.

M. Thomas has inaugurated a method of vaccination by the employment of threads impregnated with virus and passed as a seton beneath the skin of the tail. M. Darbot has thus described it.—

"This method, which has been in vogue in Haute Marne for five years, under the auspices of the veterinary society of this department, consists in placing at the extremity of the tail, through the tissues several threads rolled together, methodically impregnated with attenuated virus. The virus is renewed by passing it through the frog; attenuation is obtained by heat, and the division of the doses with which the threads are charged is effected by means of a specially constructed apparatus. The inoculation is made with a simple suture needle by simp-

ly passing the threads through the tissue. The method bears favorable comparison with others, the accidents to which it gives rise are *nil*. It is therefore safe, and the security is due to the fact that the virus is regenerated in the frog, and does not become again exalted after attenuation.

Inoculation in minimum doses is absolutely certain, the virus being deposited in a wound which cannot heal; owing to the presence of the thread its multiplication is assured. One inoculation is sufficient."

M. Eloire of Capelle adopted this method, but found seventeen animals out of fifty-eight died; he therefore abandoned it, and counsels others to do so.

M. Roux has succeeded in conferring immunity by injecting the soluble substances secreted by the microbe.

When we inject 40C.C daily of a culture of charbon symptomatique made in veal broth, and sterilized by heating for fifteen days at a temperature of 115°C., or by filtration through porcelain, into the peritoneum of a guinea pig, we confer immunity, even against $\frac{1}{5}$th centimetre cube of intensified virus, whilst control animals die from this injection. The liquids filtered through porcelain appear more active than those heated to 115°C. The serosity taken from an animal dead of the disease, filtered through porcelain and injected into the guinea pig in doses of 20C.C., renders them ill, but does not kill them, moreover, we confer immunity on the guinea pig by a daily injection of 1C.C. of filtered serosity for twelve days.

According to Strebel, Professor Kitt of Munich, not liking the tail as a seat of the inoculation, made experimental inoculations into the shoulder behind the extensors of the arm, with an energetic vaccine, and noted that these were not followed by any untoward accidents. Seeing the good results obtained, Suchanka, Palla and Schwaiger of the Duchy of Salzbourg (1888) tried vaccination on forty-three animals with the second vaccine of Lyon. In the year 1889 they inoculated thirty-three animals in the shoulder; in 1890, 1,176, one of which died four days afterwards. In 1891, 1,254 beasts were inoculated in the same way without any accident. Out of 2,506 vaccinations made with strong virus on the side of the thorax, one fatal case of charbon was noted, which result may be considered to be very favourable when we take into consideration the fact that the 2,505 animals were immune, although they had only undergone one inoculation. Considering these encouraging results, and after consulting with M. Arloing, Strebel began vaccinating in the region of the shoulder in the spring of 1893, using a special syringe having a large bore needle. He remarks as follows:—

"I inoculated twice at the shoulder 530 young cattle, 119 with the first vaccine, 198 with the second, and 213 first with the first and then with the second vaccine of MM. Arloing, Cornevin and Thomas. The total

number of inoculations at the shoulder was 743. I vaccinated 729 animals in front of the articulation of the left shoulder, eight behind the articulation of the same side, and six in the skin covering the external face of the scapula.

I had already vaccinated 370 subjects without the slightest accident, the only lesions being the formation in some cases of an œdematous tumors, and these results had given me faith and confidence in the method; then the first accident occurred, one animal succumbed and another followed. I modified my method slightly, and made 255 inoculations more. I had been employing non-filtered vaccine; it was now filtered."

Suchanka, Palla and Schwaiger, although they did not filter their vaccine, only had one case of charbon S. out of 2,506 inoculations. According to *Zimmermann* 3,666 animals were vaccinated in the Corarlberg and only eight died (0,25 per cent of the disease later on, but there was no death from inoculation.

During the spring of 1892, 4,820 animals of the same country were inoculated.

MM. Guillod of Etain and Sunon at Spincourt vaccinated 1,500 animals on the chest in the department of Meurthe-et-moselle without any accident. One animal died later of the disease (Rec de med. vet).

Strebel concludes that, despite the two fatal cases, he shall continue to vaccinate fearlessly with a slightly filtered emulsion. The advantages of his method over others are that the operation is rapid and simple, it may be made before, behind, or upon the shoulder. Forty to forty-five animals may be vaccinated in the hour. It is not necessary to cut the hair, nor to disinfect the seat of inoculation; the skin is soft; as soon as the needle is removed, the wound closes at once, and nothing can get into it. Reflux of the inoculated material does not take place. There is no hæmorrhage. The accidents to which it gives rise are not half as numerous as those following inoculation at the tail.

Out of 8,241 animals surviving the vaccination, ten only have subsequently been attacked with charbon, 0·12 per cent, whilst with the tail method the losses were triple that percentage. Out of 2,241 animals, inoculated with vaccine of Lyon only one succumbed.

Technique of Strebel's method.—The material is injected subcutaneously, care being taken to avoid wounding the flesh, and this is best effected by raising a fold of the skin between the fingers and then making the injection. (Journal med. vet. et de zoot, 1893.)

Delamotte.—Contributes a paper to the Repertoire Veterinaire, 1893, on the above subject and adds further successes to the method.

Protective inoculation in Switzerland, Austria and Prussia.—*Strebel* gives the following details of preventive inoculations against this disease.

Switzerland.—'In the spring of 1887 twenty-four thousand six hundred and fifty young cattle were inoculated. The inoculations were divided amongst the cantons as follows:—

Berne	... 14,890	Fribourg	... 1,725	Uri	... 323
Graubunden	.. 3,292	Saint Gall	... 963	Obmalden	... 180
Waadt	... 2,396	Glaris	... 707	Wallis	... 119

Of this number 132 died from the disease or 0·45 per cent. The results of the inoculations varied in the different cantons. The total losses amongst animals were as follows:—

	Per cent.
Saint Gall	0·20
Fribourg	0·23
Glaris	0·25
Berne	0·37
Waadt	0·54
Granbunden	1·
Obmalden	1·43
Uri	2·47

There was no death in Wallis. The deaths amongst inoculated animals was 1·43 per cent. altogether.

Austria.—In the spring of 1877 inoculation was carried out in the duchy of Salzbourg, in the Tyrol, the Boralberg, Caranthia and the principality of Lichteinstein with the following results:—

Duchy of Salzbourg 2,472 inoculated, eight died, or a 0·32 per cent, whilst out of 3,561 non-inoculated animals inhabiting the same pastures the losses were 225 or 6·30 per cent.; out of the eight fatal cases two had only received one inoculation.

In the Tyrol 2,183 were inoculated and thirteen succumbed to the disease. Amongst 7,499 non-inoculated animals there were 99 deaths or 1·20 per cent. Borarlberg, 1,238 inoculated, four deaths or 0·32 per cent.; amongst the non-inoculated the deaths were 9·60 per cent.

Lichteinstein inoculated 230, no death, Carinthia inoculated 221, one death. The total result shows an average 0·54 per cent. of casualties amongst the inoculated.

Prussia.—Gueldre inoculated 485, two deaths.

Cleve inoculated 180 two deaths.

Résumé. There were twenty-six deaths amongst the inoculated animals or a total of 0·86 per cent., whilst 336 non-vaccinated animals died or a proportion of 2 per cent. (Schweizer archiv., 1889, H. 1.)

Diagnosis.—The diagnosis of this disease when it is characterized by the crepitant and sonorous tumor is easy. Moreover, microscopical examination and the inoculation of the products of the tumor into guinea pigs allows of an absolutely certain diagnosis being made. When the tumor is not present, an attentive study of the lesions, the examination of diseased organs (spleen, ganglia, etc.) under the microscope, as well as the inoculation of their pulp permits of our making it. Much assistance

can be gained from the history of the case. In practice charbon symptomatique may be most commonly confounded with charbonous fever (anthrax) or septicæmia, notably gaseous septicæmia, and sometimes with gangrenous coryza. Despite its resemblance to these maladies, the above procedure enables us to distinguish it easily. With regard to septicæmia, if we inoculate a rabbit, an animal refractory to charbon symptomatique but susceptible to septicæmia, the differentiation is soon made. With regard to charbonous fever, we have a large choice of methods. The symptoms and lesions are markedly different; in the one we have crepitant tumors, in the other tumefactions which are non-crepitant. In charbonous fever the blood is very virulent, whilst in charbon symptomatique the tumor is more virulent. The first is inoculable with very small doses, whereas the other is not. The one is easily inoculated with slight punctures with a lancet, the other is not. The subcutaneous inoculation with anthrax produces a more or less insignificant local lesion, whilst that with charbon symptomatique produces the large characteristic crepitant one of that disease. Charbonous fever is easily inoculable by intravenous injection, whilst C. Symptomatique is so induced with difficulty; the first has a longer period of incubation; its microbe is "ærobic," it does not form spores in the sick, and presents different characters to that of the latter, which does form spores in the sick and is anærobic. Again, anthrax is inoculable to the rabbit, whilst C. Symptomatique is not.

Treatment.—The treatment of this disease has so far been unattended with favorable results and various remedies have been tried with little effect. Such agents as chlorate of potash, hyposulphite of soda, etc., have been used. The direction in which treatment should be adopted appears to be to attack the tumor and make injections of antiseptics into its depth, at the same time giving chlorate of potash internally.

Setoning, etc., as a preventive, is adopted in England, but the results cannot be vouched for. Information regarding experimental treatment is required.

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